Medical and Pediatric Oncology 28:136–138 (1997)

A Childhood Fibrolamellar Hepatocellular Carcinoma With Increased Aromatase Activity and a Near Triploid Karyotype

Manuela A. Hany, MD,[1]* David R. Betts, BSc,[1] Markus Schmugge, MD,[1] Eugen Schonle, MD,[2] Felix K. Niggli, MD,[1] Milo Zachmann, MD,[2] and Hans-Jurg Pluss, MD[1]

We report a 15-year-old boy with hepatocellular carcinoma (HCC) of the fibrolamellar type. He presented with advanced disease and a nonresectable tumor. Clinical features included marked gynecomastia which had been present for 3 years, failure to enter puberty, and failure to thrive. These features might have been due to a high aromatase activity of the tumor. The course of the illness suggested that the tumor had been present for at least 3 years prior to diagnosis. At diagnosis the patient had multiple metastases which included infiltrated ascites. Cytogenetic analysis of the ascites revealed a near triploid karyotype with cell-to-cell variation and an abnormality of chromosome 1q. This to our knowledge is the first karyotype report of fibrolamellar HCC in a child. **Med. Pediatr. Oncol. 28:136–138** © 1997 Wiley-Liss, Inc.

Key words: fibrolamellar hepatocellular carcinoma; aromatase; feminization; gynecomastia; cytogenetics; near triploidy

INTRODUCTION

Hepatocellular carcinoma (HCC) is a common adult tumor but one that is rare in childhood. The prognosis is poor for all age groups [1]. Typically, the age at diagnosis in children is between 5 and 15 years and there is a male prevalence [2,3]. Although the precise causes of HCC are not yet clear, there exist some well-known predisposing factors, such as persistent hepatitis, virus infection, and exposition to mycotoxins [4–6]. The majority of adult HCCs are related to chronic liver disease. Hepatocarcinogenesis is thought to be a multistep process that involves genetic alterations leading to activation of cellular oncogenes or to inactivation of tumor-suppressor genes [5,7,8]. In particular, loss of heterozygosity of 1p has been proposed to be an early event in hepatocarcinogenesis [7,9].

Causes of childhood HCC are not as well defined, although, in a small number of cases, correlation with underlying liver abnormalities can be found (e.g., hepatitis, cirrhosis, familial cholestasis, tyrosinemia) [2–4] but cytogenetic alterations have not been reported yet. A variant form of HCC has been recognized in children and young adults known as fibrolamellar HCC which has distinctive clinical and pathological features [10]. This tumor has been defined as a specific subtype with a relatively high rate of surgical resectability and improved overall survival compared with typical HCC.

We report the clinical features and tumor cytogenetics of a 15-year-old boy with fibrolamellar HCC.

CASE REPORT

KC, a 15-year old boy, was admitted to the University Children's Hospital of Zurich with an intraabdominal mass in the right upper abdomen. Three months prior to admission, the patient had fatigue and loss of appetite, but was otherwise symptomless. At presentation, his weight was 32 kg and height was 148 cm, both measurements falling below the 3rd percentile. He showed marked, symmetric gynecomastia (Tanner stage III), pubic hair was Tanner stage II, and his genitalia was Tanner stage I. Review of his past history showed that from the age of 12 onwards he fell below the expected percentiles and gynecomastia appeared at the same time.

Further investigations showed that the patient had an extended tumor, deriving from the liver, with infiltration of the lower right lung. There were multiple abdominal and lung metastases. Other clinical features were ascites and marked hepatosplenomegaly. A needle biopsy revealed fibrolamellar HCC.[1] Liver enzymes were slightly elevated, α-fetoprotein and β-human chorionic gonadotropin were normal. Antibodies for hepatitis A, B, and C were negative. The endocrine findings revealed normal luteinizing hormone (LH), low follicle-stimulating hormone (FSH; less than 0.5 U/l), low testosterone (0.62 nmol/l), very high estrone (568 pmol/l), and ele-

[1]The diagnosis was made by Professor J. Briner, Department of Clinical Pathology, University Hospital, Zurich, Switzerland.

[1]Departments of Oncology and [2]Endocrinology, University Children's Hospital, Zurich, Switzerland.

*Correspondence to: Dr. Manuela A. Hany, Department of Oncology, University Children's Hospital, Steinwiesstrasse 75, CH-8032 Zurich, Switzerland.

Received 10 November 1995; accepted 8 March 1996.

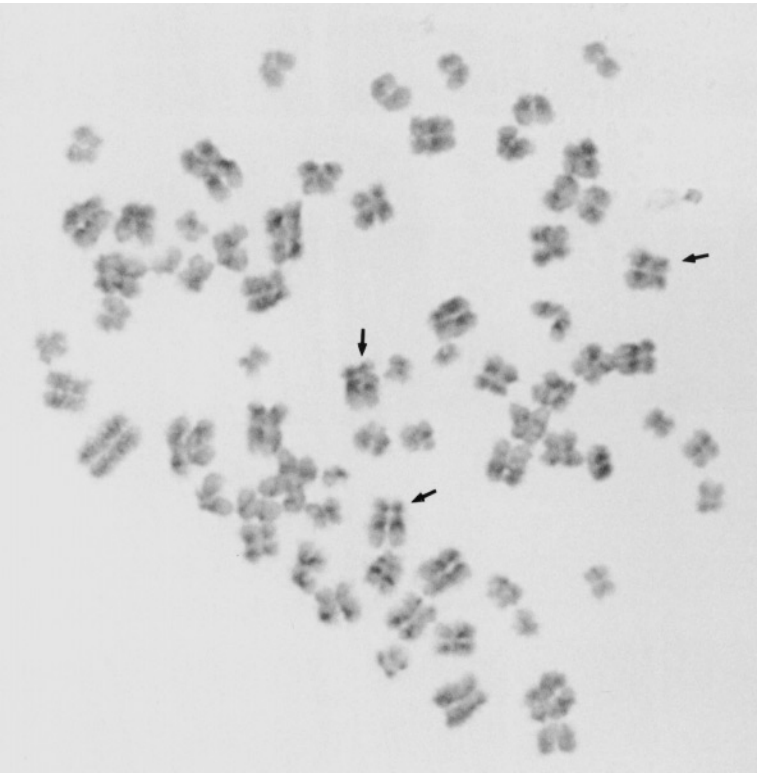

Fig. 1. A representative metaphase spread of the major clone. Chromosomes with identified chromosome 1 material are arrowed.

vated estradiol (61 pmol/l). The bone age was $15^{4}/_{12}$ years (Greulich and Pyle). Adrenocorticotropic hormone (ACTH) and dexamethasone tests demonstrated a suppression of cortisol and androstenedione and an increase in estrone.

The patient was treated with a combination of cisplatin and doxorubicin for one course. The tumor showed no response and taxol was added to the treatment. The tumor continued to respond poorly and the patient died 5 months after admission. The cause of death was liver failure with associated encephalopathy.

Cytogenetic Analysis

For cytogenetic analysis ascites was cultured and metaphases were examined according to ISCN 1991 [5]. To exclude a constitutional abnormality, a 72-hour (PHA) phytohemagglutinin-stimulated peripheral blood culture was also analyzed.

Three cell populations were seen in the ascites. The majority of cells had a 46,XY karyotype, probably representing reactive lymphocytes. The remaining cells were presumed to be from the tumor and two related abnormal clones were identified. The major clone had a karyotype of 64–72,XY,t(1;?)(q21;?),+der(1)t(1;?)(q21;?),−3,−5,−18,+der(?)t(?;12)(?;q13),+4mar,inc[cp18] with a median chromosome count of 68 [Fig. 1]. The minor clone had a count of 131–133 from six cells. The der(1)t(1;?) was identified, indicating a doubling of the major clone. Due to the poor morphology of the metaphases, further characterization was not possible. The constitutional analysis of the peripheral blood confirmed the 46,XY karyotype seen in the ascites.

DISCUSSION

From previous studies, there is evidence that fibrolamellar HCC has a better outcome than other types of HCC [3,11]. The tumor is supposed to be less malignant and will often be recognized earlier in the disease, thereby increasing the chances of tumor resection [2]. Unfortunately, in this case resection was not possible due to the advanced disease stage at diagnosis.

At the age of 12, the patient had fallen below the expected percentiles and therefore, we postulate that the tumor arose prior to this age. We consider it likely, that the tumor was producing an aromatase at this stage. Increased aromatase activity leads to a conversion of androstenedione and testosterone to estrone and estradiol, resulting in feminization and pubertal failure, as observed in this case [12]. The bone age correlated to the chronological age, demonstrating that it was not just a wasting disease responsible for the failure to thrive but also the very low

testosterone levels. A beginning adrenarche was possible due to the androgens produced by the adrenal glands.

The patient presented with symmetric gynecomastia, a finding not uncommon in liver disease; it typically results from either increased estrogen production or increased substrate for aromatase [13,14]. In common pubertal gynecomastia, the aromatase derives from the peripheral fat [14,15]. This leads only to a slight aromatase increase, still allowing males to enter puberty. The testosterone levels are still normal and reactive gonadotropin increase occurs. If the aromatase is liver derived the levels are very high, resulting in low testosterone levels and a negative feedback on the hypophyseal axis. Consequently, there is a failure to enter puberty.

Another possibility for increased estrone and estradiol levels is increased glucuronization [13,16]. Conversely, this would result in a normal testosterone level, which is not seen in our case. ACTH and dexamethasone tests indicated a nonadrenal source of the aromatase. In addition, the adrenals were small, as could be seen in the autopsy. Therefore, we may postulate that in this case the aromatase activity of the liver was high, even though the enzyme was not analyzed in the liver.

This case to our knowledge is the first report of a tumor karyotype of a child with a fibrolamellar HCC. With this case there are now six HCC tumor karyotypes in the literature [17–19], of which three have a near triploid karyotype with cell-to-cell variation. We propose that this represents a distinct cytogenetic subgroup in HCC. In both, HCC tumor and cell line [20–22] abnormalities of 1p and/or 1q are common. In this case, we identified an abnormality of 1q at q21, although given the poor morphology of the metaphases, a 1p abnormality could not be totally excluded. Further HCC karyotypic reports are required to establish the common abnormalities at the cytogenetic level.

We have demonstrated that a solid tumor karyotype can be obtained from infiltrated material other than a surgically resected piece of tumor. This will facilitate further karyotypes to establish additional common cytogenetic abnormalities in both HCC and other tumors.

ACKNOWLEDGMENTS

We thank the "Zurcher Vereinigung zur Unterstutzung krebskranker Kinder" for their financial support.

REFERENCES

1. Wands JR, Blum HE: Primary hepatocellular carcinoma. N Engl J Med 325:729–731, 1991.
2. Lack EE, Neave C, Vawter GF: Hepatocellular carcinoma: Review of 32 cases in childhood and adolescence. Cancer 52:1510–1515, 1983.
3. Farhi DC, Shikes RH, Murari PJ, Silverberg SG: Hepatocellular carcinoma in young people. Cancer 52:1516–1525, 1983.
4. Blum HE: Hepatozellulares Karzinom: molekularbiologische Aspekte. Zentralbl Chir 119:759–763, 1994.
5. Okuda K: Hepatocellular carcinoma: Recent progress. Hepatology 15:948–963, 1992.
6. Brechot C, Pourcel C, Louis A, Rain B, Tiollais P: Presence of integrated hepatitis B virus DNA sequences in cellular DNA in human hepatocellular carcinoma. Nature 286:533–535, 1980.
7. Kuroki T, Fujiwara Y, Tsuchiya E, Nakamori S, Imaoka S, Kanematsu T, Nakamura Y: Accumulation of genetic changes during development and progression of HCC: Loss of heterozygosity on chromosome arm 1p occurs at an early stage of hepatocarcinogenesis. Genes Chromosome Cancer 13:163–167, 1995.
8. Sugimura T: Multistep carcinogenesis: A 1992 perspective. Science 258:603–607, 1992.
9. Shiou-Hwei Y, Pei-Jer C, Hui-Ling C, Ming-Yang L, Chu-Chang W, Ding-Shinn C: Frequent genetic alterations at the distal region of chromosome 1p in human hepatocellular carcinomas. Cancer Res 54:4188–4192, 1994.
10. ISCN: Guidelines for Cancer Cytogenetics: Supplement to an International System for Human Cytogenetic Nomenclature. F. Mitelmann (Ed) Basel, S. Karger 1991.
11. Craig JR, Peters RL, Edmontson HA, Omata M: Fibrolamellar carcinoma of the liver: A tumor of adolescents and young adults with distinctive clinicopathological features. Cancer 46:372–379, 1980.
12. Leibermann E, Zachmann M: Familial adrenal feminization probably due to increased steroid aromatization. Horm Res 37:96–102, 1992.
13. Wilson JD: Endocrine disorders of the breast. In "Harrisons Principles of Internal Medicine," 12th ed. New York: McGraw Hill, 1991, pp. 1796–1798.
14. Carlson HE: Gynecomastia. N Engl J Med 303:795–799, 1980.
15. Nuttal FQ: Gynecomastia as a physical finding in normal men. J Clin Endocrinol Metab 48:338–340, 1979.
16. Zayed A, Stock JL, Liepman MK, Wollin M, Longcope C: Feminization as a result of both peripheral conversion of androgens and direct estrogen production from an adrenocortical carcinoma. J Endocrinol Invest 17:275–278, 1994.
17. Bardi G, Johansson B, Pandis N, Heim S, Manahl N, Andren-Sandberg A, Hagerstrand I, Mitelman F: Cytogenetic findings in three primary hepatocellular carcinomas. Cancer Genet Cytogenet 58:191–195, 1992.
18. Simon D, Munoz SJ, Maddrey WC, Knowles BB: Chromosomal rearrangements in a primary hepatocellular carcinoma. Cancer Genet Cytogenet 45:255–260, 1990.
19. Werner M, Nolte M, Georgii A, Klempnauer J: Chromosome 1 abnormalities in hepatocellar carcinoma. Cancer Genet Cytogenet 66:130, 1992.
20. Simon D, Aden DP, Knowles BB: Chromosomes of human hepatoma cell lines. Int J Cancer 30:27–33, 1982.
21. Simon D, Knowles BB: Hepatocellular carcinoma cell line and peripheral blood lymphocytes from the same patient contain common chromosomal alterations. Lab Invest 55:657–665, 1986.
22. Yoshizawa K, Kiyosawa K, Usuda S, Yabu K, Nakatsuji Y, Yamada S, Furuta K, Tanaka E, Sodeyama T, Furuta S: New hepatocellular carcinoma cell line SUHC-1 established from a patient with hepatitis C virus RNA in serum. Jpn J Cancer Res 83:871–877, 1992.